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Effects of ELF (Extremely Low Frequency) Fields on Calcium-Ion Efflux from Brain Tissue In vitro

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16. ABSTRACT

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Effects of ELF Fields on Calcium-Ion Efflux from Brain Tissue *in Vitro*[1,2]

CARL F. BLACKMAN, SHAWNEE G. BENANE, LINDA S. KINNEY, WILLIAM T. JOINES, AND DENNIS E. HOUSE

Health Effects Research Laboratory, U. S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

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It has been previously demonstrated that carrier waves of 50 and 147 MHz, when sinusoidally amplitude modulated at 16 Hz (ELF), can cause enhanced efflux of radiolabeled calcium ions from chick brain tissue *in vitro*. This phenomenon occurs only when the samples are exposed to specific intensity ranges of the carrier wave. Unmodulated carrier waves do not affect the ion efflux. Since the ELF signal must be demodulated from the carrier wave to be effective, a study of the efflux enhancement due to the ELF signal alone may lead to an identification of the site of demodulation, as well as provide clues to the underlying mechanism. We report here that 16-Hz sinusoidal fields in the absence of a carrier wave can alter the efflux rate of calcium ions. The results show a frequency-dependent, field-induced enhancement of calcium-ion efflux within the ranges 5 to 7.5 V/m and 35 to 50 V/m (peak-to-peak incident field in air) with no enhancement within the ranges 1 to 2, 10 to 30, and 60 to 70 V/m.

INTRODUCTION

Radiofrequency carrier waves of 50 and 147 MHz, when sinusoidally amplitude modulated at 16 Hz, can cause enhanced efflux of radiolabeled calcium ions from chick brain tissue *in vitro* (*1–5*). When the carrier waves are unmodulated or are modulated at frequencies below 6 Hz or above 25 Hz no radiation-induced change is observed. Thus the essential component necessary for this phenomenon is apparently a specific frequency range of extremely low frequency (ELF) fields, centered around 16 Hz.

Since specific frequencies of ELF fields when modulating radiofrequency carrier waves are active in causing enhanced efflux of calcium ions from brain tissue, it is important to evaluate the effects of ELF fields alone on this system. The only such study is that of Bawin and Adey (*6*), who reported a reduction in calcium-ion efflux at specific field intensities and at frequencies similar to those that are effective when

[1] Presented in part at the Third Annual Meeting of the Bioelectromagnetics Society, Washington, D. C., 9–12 August 1981.

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imposed on a 50- or a 147-MHz carrier. Corroboration of this work would allow us to investigate the difference in efflux results in the presence or absence of a carrier wave. In addition, the response of the tissue to various intensities of ELF fields could be compared to the results obtained with modulated radiofrequency carrier waves, whereby power density regions of enhanced efflux are bracketed by regions of no enhancement (*4*, *5*). Further, the use of ELF fields would avoid the confounding influence on the intensity response of carrier-wave dependence observed by Joines and Blackman (*5*, *7*). Finally, techniques would be available to experimentally delineate the demodulation process by which the modulated radiofrequency carrier waves cause changes in calcium-ion efflux.

In this study, we asked whether calcium-ion efflux from chick brain *in vitro* could be altered by ELF fields. Particular emphasis was placed on (1) identifying the effective frequency and intensity ranges, as compared with the modulated radiofrequency carrier-wave results, and (2) determining whether exposure causes efflux enhancement or reduction. We also described some techniques which reduced the variability in our sham data.

MATERIALS AND METHODS

Exposure system. Exposure to ELF fields was performed in a rectangular transmission line (Crawford Cell) available commercially (Instruments for Industry, Inc., Model CC 105S). Calculations based on transmission line theory (*8*) confirm that the transmission line operates in a predictable manner as an exposure chamber at frequencies in the 16-Hz range. Field intensities between 1 and 70 V/m (peak-to-peak incident fields in air) and frequencies between 1 and 30 Hz were generated by a Wavetek 5-MHz Phaselock Generator, Model 186. The field intensities were established by adjusting the input voltages from the generator as measured with a true rms digital voltmeter (Data Precision, Model 248) at 10× the exposure frequency just prior to exposure at the lower frequencies. This procedure was necessitated by frequency limitations in the Data Precision meter. Later tests with a Ballentine true rms voltmeter (Model 3020A) validated this approach. The frequencies were confirmed with a frequency counter (Digitec, Model 8510A). The signal was terminated in a 50 Ω, 10-W load (NARDA, Model 375 NM). Since the characteristic impedance of the transmission line is also 50 ohms, in normal operation the 50-ohm load prevents reflected waves so that only incident plane waves of electric and magnetic field travel from source to load through the transmission line. These incident electric and magnetic fields will be in orthogonal planes and their magnitudes will be related by the intrinsic impedance of air (377 ohms). For our transmission line, we confirmed this by checking the direction and magnitude of the magnetic field using a gaussmeter (Bell, Model 120). A diagram of the exposure system is shown in Fig. 1.

A proportional temperature controller was connected to either side of the transmission line and used as a closed system to circulate air, heated to 37° ± 0.5°C, past the samples. The transmission line was covered with foamed polystyrene insulation (2.54 cm thick) to minimize thermal gradients within the line. Samples used as controls were placed in a water bath which maintained the same temperature.

Materials. The brain tissues were from 1- to 7-day-old chicks (*Gallus domesticus*)

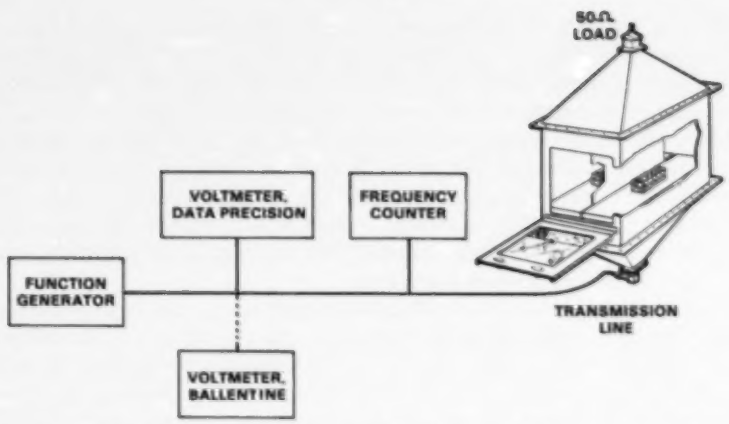

FIG. 1. Diagram of ELF exposure system. Detailed descriptions of each component are found in the text.

obtained from North Carolina State University, Raleigh, North Carolina. The medium used in this study was composed of 155 m*M* NaCl, 5.6 m*M* KCl, 2.16 m*M* $CaCl_2$, 2.4 m*M* $NaHCO_3$, and 11.1 m*M* glucose. For the radioactive labeling of tissues, this medium was supplemented with calcium-45 ($^{45}Ca^{2+}$) at 1.0 μCi/ml (New England Nuclear, 0.9 mCi/nmole). All solutions were prepared on the same day that they were used. The pH of the solutions was 7.8–8.0 and the temperature was 37°C.

Preparation of tissues. The chicks were killed by decapitation. The top of the skull and then the forebrain were removed from each chick. The hemispheres of each forebrain were maintained as an exposure–control pair throughout subsequent treatments and analyses.

After the removal of four forebrains, each one was divided at the midline and both halves were placed as a brain-tissue pair in a test tube containing 2 ml of $^{45}Ca^{2+}$-labeled medium; all tubes were then placed in a 37°C water bath and incubated for 30 min. Following this labeling period, the radioactive solution was aspirated and the tissues were rinsed in the following manner: 2 ml of unlabeled medium were added to each tube and then poured with the tissues into small plastic sieves held in a rack. The tissues were then rinsed by immersion in two 250-ml volumes of unlabeled medium. Each brain half, free of any loosely associated radioactive calcium, was then placed in a polystyrene tube (17 × 40 mm) and 1 ml of medium was added. The tubes were then sealed with latex stoppers.

Exposure of tissues. Lucite racks holding the tissue samples were arranged symmetrically on either side of the transmission-line center conductor. All of the brain samples were within the calculated uniform field regions (approximately 5 cm wide) between the parallel plates of the transmission line (Fig. 2). The axis of propagation was incident on the hemispherical bottom of the test tubes, which were positioned vertically in the line. Racks holding tubes containing the control tissues were placed in a 37°C water bath.

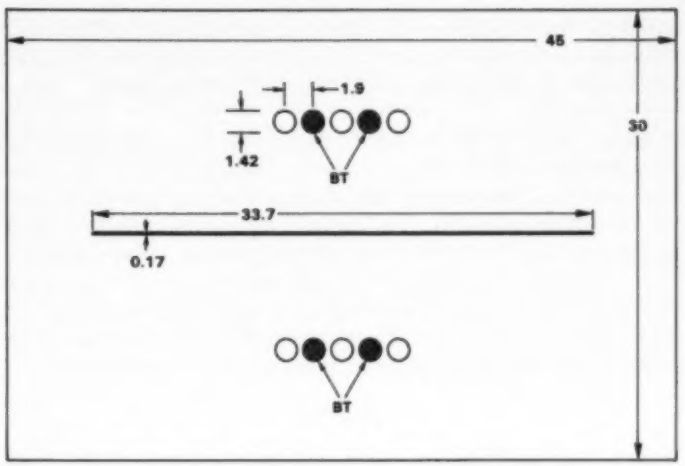

FIG. 2. Cross-sectional view of the transmission line looking down on the tops of the test tubes. The direction of propagation is upward, toward the viewer. Tubes containing brain tissue are darkened. Dimensions are in centimeters.

Four chick brains were treated at one time, with half of each brain being used as a control and the other half as an exposed sample. Sham exposures were performed in a similar manner within the transmission line with the power off.

This combined series of eight brain pairs, prepared and treated within 1 hr, represents one replication of the basic experiment. Six to nine replications were done for each combination of frequency and field intensity. Data for each combination of frequency and field intensity were completed before a new combination was examined.

The tissues were exposed to 16-Hz fields for 20 min in a series of experiments at electric field gradients of 1, 2, 3.5, 5, 6, 7.5, 10, 20, 30, 35, 40, 45, 50, 60, or 70 V/m peak-to-peak incident fields, in air. Additional exposures were conducted at 1 and 30 Hz using 40 V/m. A 10-tube exposure configuration was used for all experiments with the six additional tubes containing only an equivalent amount of unlabeled medium as described previously (*3*).

Assay for calcium-ion efflux. To measure the quantity of radiolabeled calcium ions that had been released by each tissue during the treatment period, 0.2-ml aliquots of bathing solution were added to 5 ml of scintillation cocktail (ACS, Amersham/Searle; or Liquiscint, National Diagnostics) and counted in a liquid scintillation counter.

Analysis of the data. The relative quantity of calcium ions released by each tissue pair was defined as the ratio of the counts per minute in the treated compared to the control sample (V_t/V_c).

Each combination of frequency and intensity had several replications, so the first model investigated had replication, exposure-group, and replication by exposure-group interaction effects. This model was analyzed by an analysis of variance for

a two-way design which tested for the significance of each of the three effects. None of the combinations of frequency and intensity had a significant replication or interaction effect. The model for each combination was then reduced to one with only an exposure-group effect. This model was analyzed by an analysis of variance for a one-way design which tested the significance of the exposure-group effect.

Thermal control of samples during treatment. Two procedures were used to maintain thermal stability of the samples: latex stoppers were placed on all tubes during exposure to minimize evaporative cooling, and the tubes holding the control brain halves were placed in a 37±0.5°C water bath to provide a more uniform thermal environment. The bath temperature was routinely monitored with thermocouple sensors throughout the course of these studies and adjusted as necessary to maintain this tolerance. Figure 3 shows the temperature profiles of representative samples in the transmission line and in the water bath during the 20-min exposure period.

RESULTS

Table I gives the sample size (n), mean, and standard error of the mean (SE) of V_i/V_c for the sham and exposed groups for each combination of frequency and field intensity. The P value for the one-way analysis of variance is given in the last column of the table. There was a significant ($P < 0.05$) increase in the mean V_i/V_c of the exposed group as compared to the sham group for seven of the combinations of frequency and intensity. These were all at 16 Hz and at 5, 6, 7.5, 35, 40, 45, and 50 V/m. The means at 6 and at 40 V/m were significantly different from their respective controls, each at $P \leq 0.01$.

Table I also displays the results of treatments with 1- and 30-Hz fields at 40 V/m, the intensity that gave the largest field-induced change at 16 Hz. Both frequencies produced insignificant enhancement of calcium-ion efflux ($P > 0.28$).

Figure 4 is a graph of the same data showing the mean V_i/V_c for 16 Hz as a function of incident field intensity. The bars represent one standard error. The results

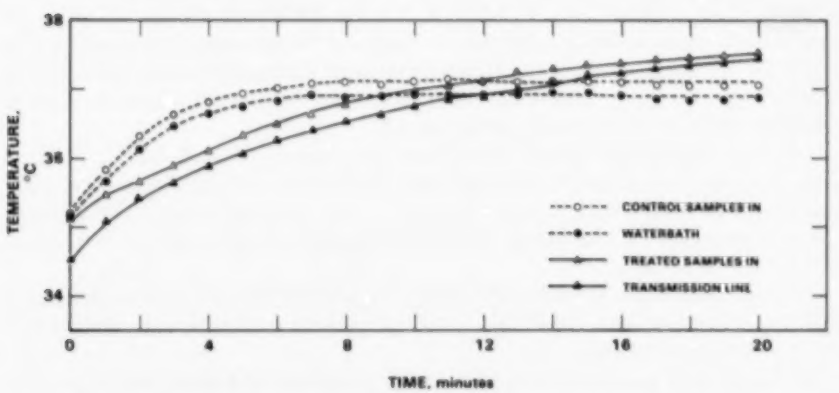

FIG. 3. Typical time–temperature profiles of treated samples in the transmission line and of control samples in the water bath during the treatment period.

TABLE I
Mean Relative Quantity of Calcium Ions Released by Brain Tissue Pairs as a Function of Exposure to ELF Fields

		V_s/V_c						
		Sham			*Exposed*			
Frequency	*Intensity (V/m)**	*n*	*Mean*	*SE*	*n*	*Mean*	*SE*	*P*
16	1	32	0.966	0.045	32	0.973	0.043	0.903
16	2	36	1.010	0.033	36	1.034	0.034	0.612
16	3.5	32	0.955	0.037	32	1.051	0.034	0.061
16	5	32	0.955	0.045	32	1.095	0.043	0.028
16	6	28	0.970	0.057	28	1.210	0.070	0.010
16	7.5	32	0.990	0.040	32	1.119	0.044	0.035
16	10	32	1.007	0.042	32	0.972	0.033	0.518
16	20	24	1.004	0.042	24	0.995	0.055	0.889
16	30	27	1.025	0.063	28	1.003	0.039	0.775
16	35	32	0.965	0.063	32	1.176	0.064	0.023
16	40	28	0.985	0.070	27	1.303	0.087	0.006
16	45	32	0.987	0.041	32	1.177	0.047	0.003
16	50	28	0.906	0.042	28	1.129	0.065	0.006
16	60	28	1.034	0.053	28	1.040	0.039	0.921
16	70	32	1.015	0.033	32	1.072	0.035	0.237
30	40	28	1.009	0.036	28	1.070	0.044	0.282
1	40	28	1.011	0.051	28	1.034	0.048	0.735

* Peak-to-peak.

of our attempt to reduce the variation previously observed in our sham exposures can be seen in this figure. The mean V_s/V_c for the sham exposures is relatively consistent over the range of intensities used.

DISCUSSION

This study was designed to investigate whether ELF fields could alter the efflux of calcium ions from chick brain tissue *in vitro* in a manner similar to that determined for modulated radiofrequency carrier waves at 50 and 147 MHz (*2–5*, *7*). The results demonstrated that 16-Hz fields cause enhanced efflux while similar intensity 1- or 30-Hz fields do not, in accordance with our previous findings, using ELF-modulated 147-MHz carrier waves (*2*). Thus the same ELF-frequency specificity is present in experiments using either type of radiation.

The intensity series of 16-Hz fields produced two well-defined intensity regions of efflux enhancement bracketed by equally well-defined regions of no enhancement. This result is similar to the two regions of efflux enhancement bracketed by no enhancement observed for 16-Hz modulated 50- and 147-MHz radiofrequency carrier waves (*4*, *5*, *7*), and thus eliminates the possibility that the radiofrequency carrier wave is the responsible agent. The appearance of multiple intensity regions of enhanced efflux bracketed by no-enhancement regions seems to be a general property of this phenomenon and provides strong evidence against any potential mechanism of action that invokes generalized heating as the underlying cause.

A comparison of the *external* electric-field intensities of ELF and modulated radiofrequency signals that produce enhanced efflux reveals that they are effective within the range of 40 to 150 V/m. However, a detailed evaluation of this apparent similarity cannot be made without an understanding of the different roles played by the radiofrequency carrier and the modulating ELF signal. In our model, the results obtained at carrier frequencies of 50, 147, and 450 MHz were reconciled only when the independent variable was taken to be the average *internal* electric-field intensity of the carrier wave (*5*, *7*). However, carrier waves without the ELF modulation did not alter the efflux of calcium ions from brain tissue (*1–3*). Thus, for the modulated carrier waves to be effective, the modulating ELF signal must be demodulated from the carrier wave. A mathematical analysis describing a possible demodulation process has been published elsewhere (*5*). In this model, it was assumed that the efficiency of the demodulation process, although unknown, would remain the same for the range of carrier frequencies under study. If this assumption is true, then the ELF signal would be present in the tissue at some unknown intensity, which would be a function of the intensity of the carrier wave. A demodulation efficiency was estimated by assuming that the internal electric-field intensity for the 16-Hz signal was the same whether it originated from the modulated carrier wave or from a directly applied ELF signal similar to the one used here. This value calculated from experimental data was 10^{-5} (*7*), although it could easily range to 10^{-7}, depending upon the permittivity value selected for brain tissue at 16 Hz. Thus we believe that without specific knowledge of the efficiency of the demodulation process, agreement between *external* electric-field intensities that induce efflux at radiofrequencies and at ELF may be more apparent than real.

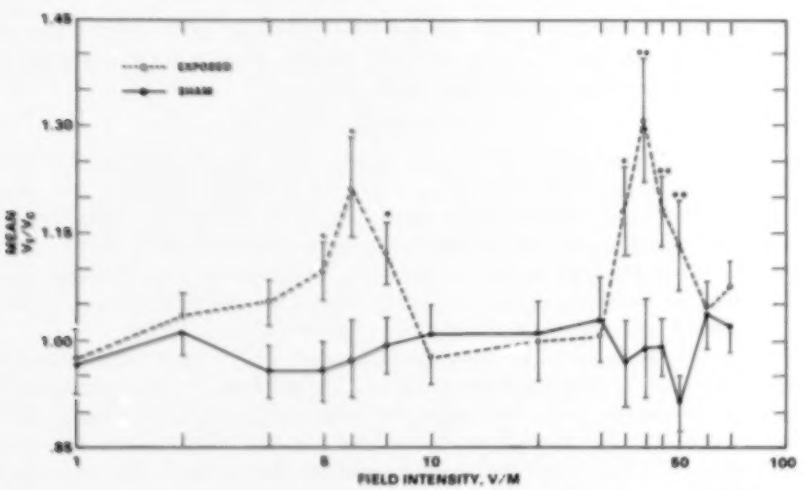

FIG. 4. Mean relative quantity of calcium ions released by tissue pairs exposed to 16-Hz fields, as a function of electric-field intensity in volts, peak-to-peak. Four tubes containing brain tissue were exposed at a time. The bars represent one standard error. Probability differences between exposed and sham groups are represented by * for $P < 0.05$ and ** for $P < 0.01$.

An additional feature of the intensity response is the narrower range of effective intensities around the lower peak-intensity value. This fact is somewhat obscured by the use of the logarithmic scale for intensity in Fig. 4. The widths of these effective intensity ranges appear to be proportional to the intensity values at the enhancement peaks. If this relationship holds for lower intensities that may cause efflux enhancement, care must be exercised in future investigations to avoid missing the narrower regions of effective intensities.

The final comparison with modulated radiofrequency radiation is whether ELF fields produce enhancement or reduction of calcium-ion efflux from chick brain tissue *in vitro*. The data demonstrate efflux enhancement in agreement with our results using modulated 50- and 147-MHz radiation. This result indicates that study of the demodulation process can be initiated without the encumbrance of opposing efflux directions for the two types of signals.

It is useful to compare our results with the only other study of ELF field-induced calcium-ion efflux from chick brain tissue *in vitro* reported by Bawin and Adey (*6*). The frequency specificity we found for radiation-induced efflux alteration, that is, significant change at 16 Hz but no change at 1 or 30 Hz, agrees with their finding of significant alterations at 6 and 16 Hz, and none at 1 or 30 Hz. Thus we have corroborated the frequency specificity they reported.

Our results agree, in a general way, with their finding of a single intensity region of altered efflux bracketed by intensities that produce no change in efflux. In contrast, however, our more detailed intensity series revealed two ranges of altered efflux, each bounded by regions of no alteration. Moreover, the intensities found for peak alteration by 16-Hz fields, 6 and 40 V/m, do not agree with the values of 10 and 56 V/m reported by Bawin and Adey. In fact, our results at 10 V/m definitely indicate no efflux alteration, while our preliminary results at 56 V/m (not shown) also indicated no alteration ($P = 0.185$). Our exposure system contained both an electric and a magnetic field, while the Bawin–Adey exposure system was basically electric and with only a small magnetic component. Based on calculations by Spiegel (*9*), the magnetic-field component in our exposure system would induce an electric field in the sample that is less than 7% of the intensity caused directly by the corresponding electric-field component. Thus the cause of this discrepancy in effective intensities is unknown. However, Bawin and Adey's results can be made to coincide with ours if it is assumed that their estimates of intensity are too high by 30 to 50%. In any event, they made no attempt to locate the maximally effective intensity as we have done.

The question of whether ELF fields can cause a reduction in calcium-ion efflux as reported by Bawin and Adey (*6*) or an enhancement as reported for radiofrequency carrier waves modulated at ELF (*1–5, 10*) is the most apparent point of disagreement between the two studies. Our experimental results unequivocally demonstrate enhanced efflux induced by various intensities of 16-Hz fields in direct opposition to the diminished efflux induced by similar fields as reported by Bawin and Adey. We currently have no definitive explanation for this difference. The cause may be found in the slightly different preparations and procedures used in the two laboratories. Although the exposure systems used in the two studies are different, if the intensity of the electric-field component is assumed to be the fundamental variable, then as

described above, the magnetic-field component in our studies produces only a slight perturbation in the overall electric-field intensity and would not account for the different results. Because internal electric-field intensities have been successfully used to reconcile differences in results obtained with various radiofrequency radiations (*5*), it is reasonable to expect this variable to be of fundamental importance in studies with ELF radiation as well. This discrepancy in the direction of efflux change will probably be resolved once the mechanism responsible for the ELF-induced efflux changes is understood. In any event, our finding that 16-Hz fields can cause enhanced efflux is in agreement with our previous results of enhanced efflux using 50- and 147-MHz carrier waves modulated with ELF, and with the results of Bawin and Adey and of Sheppard *et al.* using 147- and 450-MHz similarly modulated carrier waves. Whether applied directly or as a modulation of a carrier wave, the 16-Hz signal seems to be the essential factor which causes enhancement of calcium-ion efflux.

We carefully controlled the temperature of the samples during the treatment period to minimize the possibility that gross temperature rise could be the cause of the efflux enhancement. Calculations have shown that exposure to modulated 50- and 147-MHz radiation produces less than a 0.0005°C rise in the temperature of the samples (*5*). Measurements with a nonperturbing temperature probe (Vitek) have supported this estimate. The ELF-field intensities used in this study are calculated to produce substantially less heating. Thus efflux enhancement is not caused by generalized heating of the samples.

To further minimize temperature fluctuations in the samples during treatment, two procedures were used: the tubes were sealed with stoppers, thereby eliminating the influence of evaporating cooling, and the control samples were placed in a thermostated water bath during exposure. The water bath provided a more efficient exchange of heat and a more constant thermal environment for the samples. The temperature profile of the treated and control samples shown in Fig. 3 indicates that the control samples reach a final, limiting temperature more rapidly than the treated samples. Since we have shown that increased temperature promotes more efflux (*4*), the control samples display slightly more efflux than their sham-exposed mates, thus causing the V_s/V_c ratio to be slightly less than 1.0. This slight temperature difference is the same for both sham and exposed groups. Moreover, because of our experimental design this difference does not affect the overall results.

We have reported multiple power density ranges of modulated 50- and 147-MHz radiation to be effective in enhancing the efflux of calcium ions from brain tissue (*4*, *7*). In these reports, the ranges frequently consisted of only one data point, separated by similarly documented no-enhancement ranges. Although the labor-intensive nature of these experiments requires judicious selection of exposure parameters to optimize the extent of values investigated, we elected to maximize the detail within a narrow intensity range to provide unequivocal evidence for existence of multiple intensity windows. We believe this study using ELF fields does provide such evidence and, in so doing, supports similar observations made in previous papers addressing modulated radiofrequency carrier waves (*4*, *7*). Although only two ranges of enhanced efflux were identified in this study, the existence of more ranges at either higher or lower intensities cannot be precluded.

Confirmation and extension of the concept of multiple intensity ranges for radiation-enhanced calcium-ion efflux (separated by no-effect regions) have distinct implications for the design of future *in vitro* and *in vivo* experiments. When low-level effects are suspected at any particular intensity of electromagnetic radiation (EMR), additional experiments should be performed at slightly higher and lower intensities to maximize the opportunity for detection of EMR-induced responses. This approach may be especially advantageous in experiments requiring substantial efforts to detect positive responses within a noisy background. For example, Gavalas-Medici and Day-Magdaleno (*11*) reported that ELF fields at 10 and 56 V/m, of frequencies similar to those used in the calcium-ion efflux studies, could cause a statistically significant change in the interresponse time of monkeys trained to respond to a stimulus. An intensity of 1 V/m had no effect. If these workers had examined the response to intensities slightly higher and lower than the effective ones, it is possible that they would have observed an even stronger response in the animals. Thus the concept of multiple intensity ranges may provide some guidance for future experiments to extend their work and for other *in vivo* studies.

Of course, it is also possible that the occurrence of multiple intensity ranges of effective EMR is peculiar to our particular sample and/or exposure situation. Since this pattern of effects as a function of intensity has been observed in two distinctly different Crawford cells and at frequencies approximately 10^6 Hz apart, it seems unlikely to be equipment dependent. It would be useful to have independent confirmation of these results, in order to allow a better assessment of the ramifications for other experimental approaches.

The significance of these *in vitro* findings for the intact animal has not yet been determined. The observed frequency specificity is in the same range as frequencies recorded in the electroencephalogram from living animals. This correlation provides a convenient basis for assuming that the results have some origin in the neurophysiology of intact neural tissue. The basis for the response to intensity is unknown. More detailed discussions of this topic can be found elsewhere (*5*, *12*, *13*).

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REFERENCES

1. S. M. BAWIN, L. K. KACZMAREK, and W. R. ADEY, Effects of modulated VHF fields on the central nervous system. *Ann. N.Y. Acad. Sci.* **247**, 74–81 (1975).
2. C. F. BLACKMAN, J. A. ELDER, C. M. WEIL, S. G. BENANE, D. C. EICHINGER, and D. E. HOUSE, Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: Effects of modulation frequency and field strength. *Radio. Sci.* **14**, 93–98 (1979).
3. C. F. BLACKMAN, S. G. BENANE, J. A. ELDER, D. E. HOUSE, J. A. LAMPE, and J. M. FAULK, Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: Effect of sample number and modulation frequency on the power-density window. *Bioelectromagnetics* **1**, 35–43 (1980).

4. C. F. BLACKMAN, S. G. BENANE, W. T. JOINES, M. A. HOLLIS, and D. E. HOUSE, Calcium-ion efflux from brain tissue: Power density vs. internal field intensity dependencies at 50-MHz RF radiation. *Bioelectromagnetics* **1,** 277–283 (1980).
5. C. F. BLACKMAN, W. T. JOINES, and J. A. ELDER, Calcium-ion efflux in brain tissue by radiofrequency radiation. In *Biological Effects of Nonionizing Radiation* (K. H. Illinger, Ed.), pp. 299–314. American Chemical Society, Washington, D. C., 1981.
6. S. M. BAWIN and W. R. ADEY, Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency. *Proc. Natl. Acad. Sci. U.S.A.* **73,** 1999–2003 (1976).
7. W. T. JOINES and C. F. BLACKMAN, Power density, field intensity, and carrier frequency determinants of RF-energy-induced calcium-ion efflux from brain tissue. *Bioelectromagnetics* **1,** 271–275 (1980).
8. S. RAMO, J. R. WHINNERY, and T. VAN DUZER, *Fields and Waves in Communications Electronics.* Wiley, New York, 1965.
9. R. J. SPIEGEL, ELF coupling to spherical models of man and animals. *IEEE Trans. Biomed. Eng.* **BME-23**(5), 387–391 (1976).
10. A. R. SHEPPARD, S. M. BAWIN, and W. R. ADEY, Models of long-range order in cerebral molecules: Effects of sub-ELF and of modulated VHF and UHF fields. *Radio Sci.* **14,** 141–145 (1979).
11. R. GAVALAS-MEDICI and S. R. DAY-MAGDALENO, Extremely low frequency, weak electric fields affect schedule-controlled behavior of monkeys. *Nature (London)* **261,** 256–259 (1976).
12. W. R. ADEY, Tissue interactions with nonionizing electromagnetic fields. *Physiol. Rev.* **61,** 435–514 (1981).
13. W. R. ADEY, Ionic nonequilibrium phenomena in tissue interactions with electromagnetic fields. In *Biological Effects of Nonionizing Radiation* (K. H. Illinger, Ed.), pp. 271–297. American Chemical Society, Washington, D. C., 1981.